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Topology of Outer Membrane Porins in Pathogenic *Neisseria* spp.

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In *Escherichia coli*, membrane-spanning amphipathic β-sheet structures are characteristic of many outer membrane proteins. By applying the principles that have been recognized for them to the four classes of neisserial porins, we have constructed a model for the topology of the porins within the outer membrane. This model predicts eight surface-exposed loops, both in the meningococcal class 1 and 2 proteins and in the gonococcal PIA and PIB proteins. The transmembrane sequences are highly conserved among these porins and are able to form an amphipathic β-sheet structure. The surface-exposed hydrophilic loops show extensive variation in both length and sequence. Experimental evidence in support of this model has been obtained by using antisera against synthetic peptides which correspond to surface-exposed loops in class 1 and 2 proteins. Thus, binding to the cell surface was observed with antibodies against loops 1, 4, and 5 of class 1 and loops 1 and 5 of class 2. In class 1, these loops are the longest ones and show the highest sequence diversity among strains of different subtypes. Mapping of epitopes recognized by monoclonal antibodies with bactericidal activity has also provided strong support for the model. The epitopes are located in loops 1 and 4 of class 1 protein, loop 5 of PIB, and loop 6 of PIA. A nonbactericidal antibody that binds only weakly to whole cells was shown to recognize loop 3 of PIB. These results suggest that the longest loops are immunodominant, provide the binding sites for bactericidal antibodies, and display the greatest variation among different strains.

The neisserial porins are among the most abundant proteins present in the outer membrane of these organisms, and unlike several other major surface antigens they do not undergo antigenic shift during infection. They are present in the outer membrane as trimers and function by creating pores through which small hydrophilic solutes can pass in a diffusionlike process (5, 19). Their universal presence in both *Neisseria meningitidis* and *Neisseria gonorrhoeae*, as well as their exposure at the cell surface, make them candidates for components of vaccines against these organisms.

Although expression of an individual porin is stable within a strain, differences occur between strains which are responsible for antigenic diversity. Thus, all meningococcal isolates contain either a class 2 or class 3 porin, which are the equivalents of the gonococcal PIA and PIB proteins (5, 19). They all exhibit differences which generate antigenic diversity between strains and form the basis for division of meningococcal and gonococcal strains into different serotypes (13, 21). Extensive sequence homology among the PIA, PIB, and class 2 proteins has been demonstrated (8, 14, 25).

The class 1 protein is expressed by almost all meningococcal isolates, although there is considerable variation in its level of expression (29). In contrast to the other well-characterized meningococcal outer membrane proteins, there is no known equivalent of this protein in gonococci. The structural gene for the class 1 protein has been cloned and sequenced (3), and it appears that this protein is structurally related to the neisserial porins. Direct evidence for a pore function for the class 1 protein was recently obtained through the use of a class 3 protein-deficient strain (39). In contrast to the anion-selective pores formed by class 2 and 3 proteins and the gonococcal porins, the class 1 protein pores were found to be cation selective (39). Antigenic diversity among class 1 proteins forms the basis for division of meningococcal strains into different subtypes (2). Monoclonal antibodies with in vitro bactericidal activity in the presence of complement have been raised against the PIA/PIB, class 1, and class 2/3 proteins (31, 34, 40, 41), thus underlining their potential as vaccine components. In *N. meningitidis*, this bactericidal activity is strongly influenced by the growth medium in the case of the class 2/3 protein but not with the class 1 protein (31). Also, in an animal model system, only the antibodies against the class 1 protein were highly protective against bacterial challenge (34), thus making this protein a prime vaccine candidate.

A comparison of three class 1 sequences corresponding to different subtype specificities reveals high homology, with major variation confined to two discrete regions (23). The epitopes recognized by monoclonal antibodies with subtype-specific bactericidal activity were localized to either one of these variable domains (23). This is in accord with previous studies with monoclonal antibodies which suggested that the class 1 proteins contain two independent subtype-specific epitopes (2). Similarly, in the case of the PIB protein, a sequence comparison of three different serovars shows two variable regions, although at positions in the polypeptide different than in the class 1 protein (6). In this case the epitopes for type-specific monoclonal antibodies have been located in the first variable region (6).

The currently available information on sequences of neisserial porins and the location of surface-exposed epitopes should allow one to construct models for the topology of the porins in the outer membrane. In the case of several *Escherichia coli* outer membrane proteins, extensive biophysical and genetic studies have demonstrated that their membrane-spanning segments form amphipathic β-sheets (38, 42). They have multiple surface-exposed segments which can be recognized in the amino acid sequences as the

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most hydrophilic and/or variable regions (38). By extending the principles recognized for the structure of *E. coli* outer membrane proteins to the neisserial porins, we have now constructed a model for the folding of the class 1, 2, PIA, and PIB proteins in the outer membrane. Experimental confirmation of the proposed structures both by immunization studies with synthetic peptides and by locating surface-exposed epitopes is also reported. The predicted structures should form a useful working model for further investigations on the vaccine potential of these proteins and peptides derived therefrom.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *N. meningitidis* 2996 has been sero(sub)typed as B:2b:P1.2 (28). Meningococci were grown on GC agar (Difco) plates supplemented with IsoVitalex overnight at 37°C in a humid atmosphere containing 5% CO_2 (30). For the preparation of outer membrane vesicles (OMV), they were grown in 40-liter fermentors in adapted Frantz medium with pH and pO_2 control to the late exponential phase (4). *E. coli* Y1090 (44) and NM522 (15), used for the propagation of phage λgt11 and plasmid pTZ19R, respectively, were grown in LB medium (33). When necessary, the medium was supplemented with maltose (0.2%) or ampicillin (100 μg/ml).

Recombinant DNA techniques. Meningococcal DNA was isolated (37) and used for the construction of a λgt11 gene bank as described previously (36). Immunological screening with monoclonal antibody MN4C5E (28) and protein A-peroxidase was carried out after overlaying the plaques with nitrocellulose filters (36). Positive plaques were amplified and phage λ DNA was isolated as described by Sambrook et al. (33). Subcloning into plasmid pTZ19R (24) was done by standard recombinant DNA techniques (33). DNA hybridizations were carried out by using the nonradioactive DNA labeling and detection kit supplied by Boehringer Mannheim according to the instructions of the manufacturer. Plasmid DNA was isolated by CsCl gradient centrifugation (33) and used for sequencing with an automated DNA sequencer (Applied Biosystems, model 370A). Sequencing reactions were carried out with T7 DNA polymerase and according to the instructions in the Applied Biosystems manual. Fluorescently labeled universal and reversed M13 primers were used to obtain sequences from subclones carrying different restriction fragments.

Cloning and sequencing of the class 1 protein gene from strain 2996. The λgt11 gene bank containing mechanically sheared, 1- to 8-kb *Eco*RI-linkered fragments of chromosomal DNA from meningococcal strain 2996 was screened with monoclonal antibody MN4C5E, which is specific for class 1 protein of subtype P1.2. Two immunoreactive clones were isolated, both of which were found to carry a 1.9-kb *Eco*RI fragment. Further restriction analysis showed that the clones were identical. Sequence analysis revealed that the cloned fragment encodes the C-terminal part of the class 1 gene, starting at the *Eco*RI site around residue 100 (3). Using this fragment as a hybridization probe, it was demonstrated that the complete gene is located on a 2.2-kb *Xba*I fragment, as has been found with class 1 genes of three other subtype specificities (23). This fragment was also cloned into plasmid pTZ19R, and from it the remaining N-terminal sequence of the class 1 gene was subsequently obtained. The complete sequence of both strands was obtained from subclones which were constructed on the basis of known restriction sites in the published class 1 sequence (3).

Peptide synthesis. A Biolynx 4170 automated synthesizer (Pharmacia/LKB) was used for continuous-flow solid-phase synthesis (10). The syntheses were performed with N^{α}-fluorenylmethoxycarbonyl amino acid derivatives (12) by standard procedures. An N-terminal *S*-acetyl mercaptoacetyl group was introduced in the last cycle of the synthesis to enable selective conjugation of the peptides (9). Removal of side-chain protecting groups and cleavage from the solid support was effected with trifluoroacetic acid in the presence of an appropriate scavenger (water, thioanisole, or anisole/1,2-ethanedithiol, depending on the protecting groups used). The *N*-(*S*-acetylmercaptoacetyl) peptides obtained were 50 to 90% pure as determined by reverse-phase high-performance liquid chromatography analysis and were used in conjugation experiments without purification.

Peptide-tetanus toxoid conjugates. A solution of *N*-succinimidyl bromoacetate (4.7 mg, 10 μmol) in *N*,*N*-dimethylformamide (100 μl) was mixed with a solution (3.5 ml) of tetanus toxoid (20 mg) in 0.1 M sodium phosphate buffer (pH 7.8). After 1 h, 1.8 ml of the reaction mixture was subjected to gel filtration on a Sephadex PD-10 column (Pharmacia), which had been equilibrated in 0.1 M sodium phosphate containing 5 mM EDTA (pH 6.1 or 7.8) (these buffers were deaerated with helium). The modified protein was collected in 3.5 ml of eluant. A solution (1.2 ml, pH 6.1) of bromoacetylated tetanus toxoid was added to 3 μmol of the *N*-(*S*-acetylmercaptoacetyl) peptide 032 (in 100 μl of 2,2,2-trifluoroethanol), 017 (in 1.0 ml of acetonitrile/buffer [pH 6.1], 1:1 [vol/vol]), 018, 027, 029, or 028b (dry powder), or 031 (in 1.0 ml of 2,2,2-trifluoroethanol). Next, 150 μl of 0.2 M hydroxylamine (in buffer [pH 6.1]) was added. After 16 h, remaining bromoacetyl groups were blocked by addition of 2-amino ethanethiol (4 μmol). After a further period of 24 h, the conjugates were purified over PD-10 columns in buffer (pH 6.1). The appropriate fractions were combined and stored at 4°C. Conjugation of peptides 030, 025b, and 024 (dry powders) and 026 (in 0.5 ml of 2% sodium dodecyl sulfate), followed by gel filtration, was performed in a similar way at pH 7.8.

Immunization and serology. Outbred 14- to 17-g female NIH mice were immunized subcutaneously with 10 μg of peptide-tetanus toxoid conjugate in saline and 0.5 mg of $AlPO_4$ in a volume of 0.5 ml. Immunization was carried out twice, with a 4-week interval. Animals were bled at week 6. Sera were analyzed in an enzyme-linked immunosorbent assay (ELISA) using either whole cells, OMV, or synthetic peptides as coat antigens. To prepare OMV, meningococci were inactivated at 56°C for 30 min, harvested by centrifugation at 3,000 × *g* for 60 min, resuspended in phosphate-buffered saline (PBS), and lyophilized. Lyophilized cells were resuspended in 10 mM Tris (pH 8.0) and ultrasonicated on ice for 15 min (Branson Sonifier, 50% duty cycle, 50% output). Cell debris was removed by centrifugation at 10,000 × *g* for 10 min, and cell envelopes were then isolated by centrifugation at 50,000 × *g* for 75 min. Clean OMV devoid of cytoplasmic membrane proteins were prepared by treatment with 1% sodium lauroylsarcosinate (sarcosyl; Sigma) and centrifugation at 10,000 × *g* for 10 min and then 75,000 × *g* for 75 min to pellet OMV. Crude membranes were suspended in 10 mM Tris (pH 8.0), and the protein content was determined by using the BCA protein assay reagent (Pierce Chemical Co.) with bovine serum albumin as a standard.

Coating of ELISA plates was carried out with round-bottom polyvinyl chloride plates (Flow) with 100 μl of protein OMV (2 μg/ml) in PBS for 16 h at room temperature.

Coating with whole bacteria was done as described previously (1). Synthetic peptides were coated as follows. ELISA plates were coated at 37°C for 30 min with 100 μl of poly-L-lysine (10 μg/ml; molecular weight 30,000 to 70,000; Sigma) made freshly in PBS from stocks of 100 μg/ml. Plates were emptied, dried, and coated with 100 μl of peptides (5 μg/ml) in PBS for 90 min at 37°C. Before addition of antibodies plates were washed twice with water containing 0.02% Tween 80 (Merck). Antisera diluted in PBS were added and incubated for 1 h at 37°C; 0.5% Protifar (Nutricia) was used to block aspecific binding. Washing was done three times with 0.02% Tween 80 in water. Rabbit anti-mouse immunoglobulins–peroxidase (homemade [26]) were used at working dilutions in PBS plus 0.5% Protifar and incubated for 1 h at 37°C. After the plates were washed three times they were incubated for 10 min at room temperature with a freshly made working solution of tetramethylbenzidine (TMB; Sigma T5513; 1.5% [vol/vol] stock TMB [6 mg/ml] in 96% ethanol, 10% [vol/vol] 1.1 mM sodium acetate [pH 5.5], and 0.025% [vol/vol] H_2O_2 [30%]). The reaction was terminated with 100 μl of 2 N H_2SO_4, and plates were read at 450 nm with a Titertek Multiscan spectrophotometer (Flow).

Monoclonal antibodies and epitope mapping. With the exception of those for SM100 and MN4C5E, the epitopes for all monoclonal antibodies mentioned in this study have been mapped previously (see Table 2). Monoclonal antibody SM100 reacts in a serotype-specific manner with gonococci expressing PIA and promotes complement-mediated killing of such strains (40). The epitope recognized by this antibody was determined by using solid support-coupled peptides. A series of overlapping peptides corresponding to the sequence of PIA from strain FA19 (8) were synthesized on polyethylene rods via a stable β-alanine link, using pentafluorophenyl esters of Nα-9-fluorenylmethoxycarbonyl-L-amino acids as previously described (23). After removal of side-chain protecting groups, the support-coupled peptides were incubated with the monoclonal antibody and washed, and immunological reactivity was detected by ELISA after reaction with peroxidase-conjugated goat anti-mouse immunoglobulin G (23).

Sequence accession number. The new sequence data presented in this paper have been submitted to the EMBL Data Library and assigned the accession number X60105.

RESULTS

Comparison of neisserial porin sequences. In Fig. 1, all currently available complete porin sequences of *N. meningitidis* and *N. gonorrhoeae* are compared: four class 1 and one class 2 protein from *N. meningitidis* and one PIA and three PIB proteins from *N. gonorrhoeae*. Among the class 1, class 2, PIA, and PIB sequences one can identify eight rather discrete, highly variable regions, which are separated from each other by stretches that are strongly conserved in all four protein classes. The homology between the porins of a single type is even higher; for example, 87% of the sequences of the class 1 proteins is identical in all four strains. The major variation is confined to two discrete regions. A similar situation is found with the three PIB sequences, but the two variable regions are located at entirely different positions.

Construction of a model for the topology of neisserial porins. The mosaic pattern of alternating constant and variable regions, as shown in Fig. 1, is similar to the situation found for *E. coli* porins. In that case, it was further demonstrated that the variable regions are located at the cell surface, whereas the constant sequences form membrane-spanning β-sheets. Thus, the model shown in Fig. 2 was constructed by applying the same principles that have been recognized for the structure of *E. coli* outer membrane proteins (see reference 38 for a review). First, the eight variable regions are placed at the cell surface, with the intervening more conserved sequences located within the outer membrane or periplasm. An additional criterion for surface exposure is provided by the location of hydrophilic maxima in a hydropathy profile (22), which in almost all cases correspond to the variable regions (Fig. 3). The membrane-spanning segments were inspected for their ability to form amphipathic or hydrophobic β strands of 9 to 12 residues; this is possible in 12 of the 16 segments in class 1 protein (Fig. 2). The second loop is flanked by two such amphipathic sequences, which constitutes another reason for assuming its surface exposure in spite of its somewhat lower degree of variability. In Fig. 2, the length of the transmembrane sequences was chosen to be 10 residues. These sequences are identified in the following way: (i) they should preferably have one side of a potential β sheet consisting entirely of hydrophobic residues, and (ii) they should consist of as many conserved residues as possible. A final criterion is provided by minimizing the number of residues at the periplasmic side. The substitutions that are found in the transmembrane sequences are almost all conservative and do not affect the potential to form a β sheet with a hydrophobic surface. The only exceptions are formed by the transmembrane strand preceding loop 3, which lacks a hydrophobic surface in class 1 protein but not in the other porins, and by the transmembrane strand preceding loop 4, where in PIA the pattern is disrupted by a single amino acid deletion.

As can be seen in Fig. 2, the model is essentially the same for all four proteins; they differ only in the lengths and sequences of the eight surface-exposed loops. Major differences in loop length are observed in class 1 protein compared with the other porins: loops 1 and 4 and to a lesser degree loop 5 are considerably longer (Fig. 4). Another prominent difference is found in loop 5, which is much shorter in PIA than in PIB or class 2 protein. With the exception of loops 2 and 7, which are not particularly variable, there is very little sequence homology between the surface-exposed segments in the four different porins.

Antibodies against synthetic peptides corresponding to surface-exposed loops. In order to test some of the predictions made in the model, peptides corresponding to exposed sequences in the class 1 and 2 proteins were synthesized, conjugated to tetanus toxoid, and used for immunization of mice. The resultant antisera were tested for reactivity with whole cells of four different meningococcal strains (Table 1). In the case of class 1 protein, antisera against peptides from loops 1, 4, and 5 were positive with whole cells, thus confirming the surface exposure of these regions. With antisera against 018 and 021, reactivity was limited to the homologous strain, whereas with antisera against 032 and 025b binding was also observed with another strain; this is in agreement with the presence of more conserved sequences in the last two peptides. With 017 antiserum, reactivity was found with strains MC50 and H44/76, which share the P1.16 epitope in loop 4. In the case of class 2 protein, peptides corresponding to loops 1 and 5 generated antibodies that bound to whole cells of three different strains; no binding was found for strain H44/76, which has a class 3 instead of class 2 protein.

When the ELISA was done with OMV as the coat antigen,

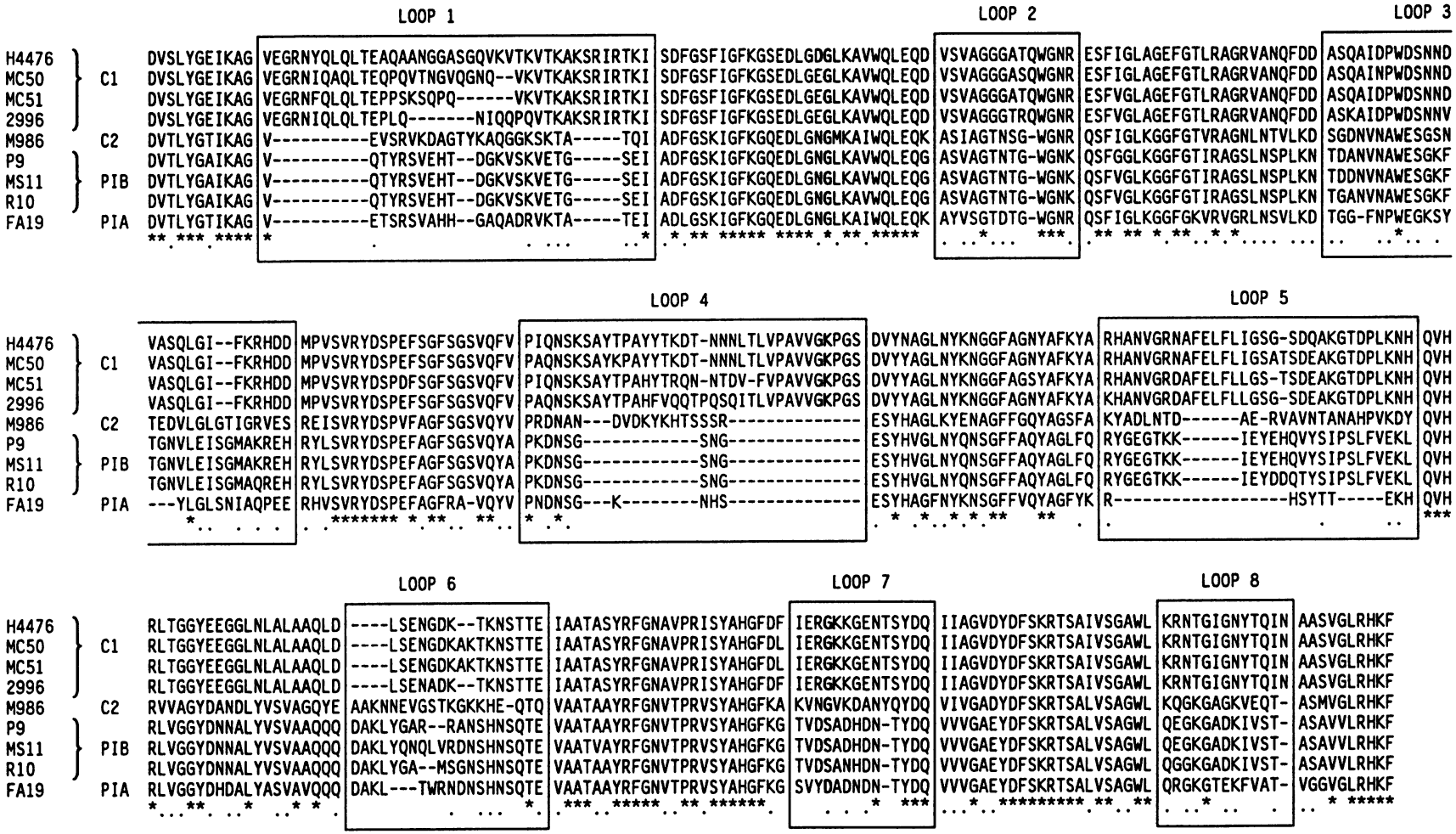

FIG. 1. Sequence alignment of neisserial porins. The class 1 sequences for strains MC50, MC51, and H44/76 have been published (3, 23); the one for strain 2996 is from this study. The class 2 (M986), PIA (FA19), and PIB (R10, P9, and MS11) sequences are from references 25, 8, and 14, 6, and 7, respectively. The boxes delineate the most variable regions and correspond to the eight surface-exposed loops shown in Fig. 2. The multiple sequence alignment was performed by using the CLUSTAL algorithm (16).

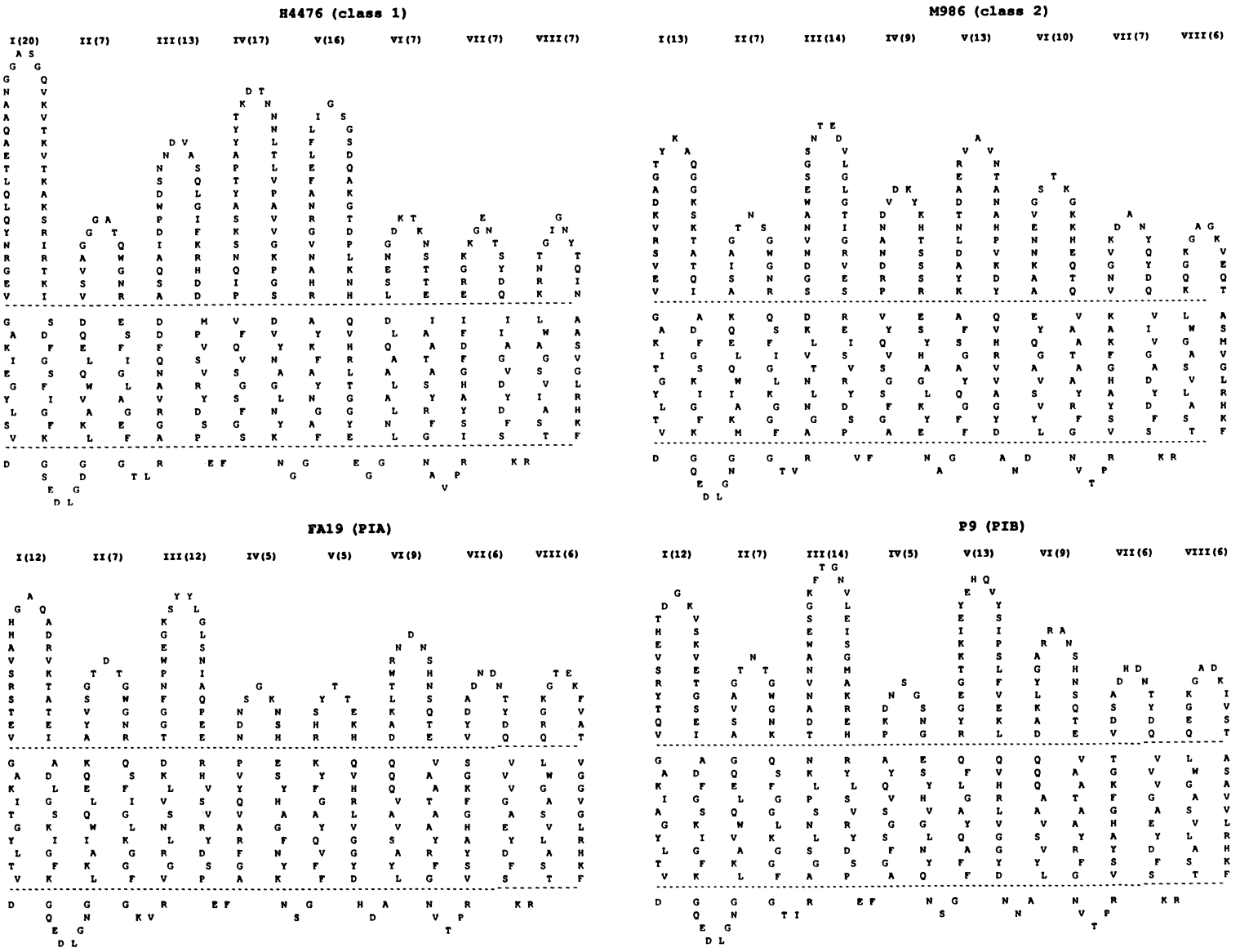

FIG. 2. Topology models of class 1, class 2, PIA, and PIB proteins. The top part of each model shows the surface-exposed regions, whereas the central part indicates the presumed transmembrane segments, whose length is chosen to be 10. Amino acid residues are shown alternating where they can form a β strand with at least one side consisting entirely of hydrophobic residues. Classification of amino acids into hydrophobic and hydrophilic was done according to the normalized consensus scale of Eisenberg et al. (11).

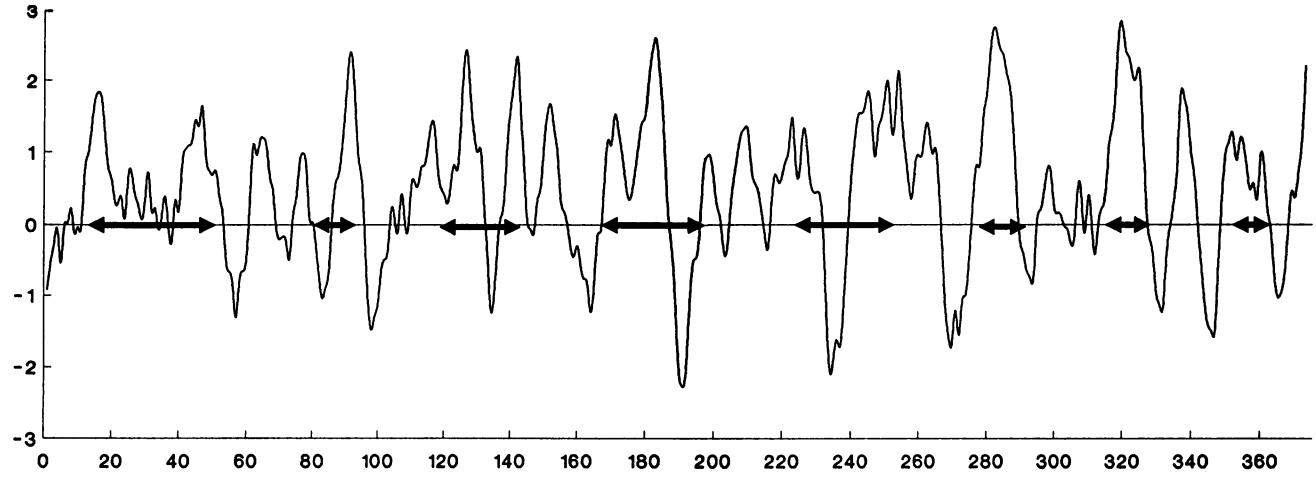

FIG. 3. Hydropathy plot of class 1 protein (strain MC50), calculated with the algorithm of Kyte and Doolittle (22). The arrows correspond to the eight surface-exposed loops shown in Fig. 2.

similar results were obtained, except with antisera against 036 and 037 (results not shown). These antisera, directed against loops 3 and 4 of class 2 protein, showed no binding to whole cells but reacted strongly with OMV from strains M986 and 2996.

Mapping of surface-exposed epitopes for monoclonal antibodies against class 1, PIA, and PIB proteins. By synthesizing peptides spanning the entire amino acid sequence, epitopes for monoclonal antibodies against class 1 (23), PIB (6), and PIA (this study) proteins have been mapped; the results are summarized in Table 2. With the exception of the PIB-specific antibody SM198, these monoclonal antibodies are all bactericidal in the presence of complement and must therefore bind to surface-exposed amino acid residues. These epitopes are located in loops 1 and 4 for class 1 protein, loop 5 for PIB, and loop 6 for PIA. Monoclonal antibody SM198, which binds only weakly to whole cells, has its epitope in loop 3 of PIB.

For the definition of the epitope recognized by monoclonal antibody SM100, a series of overlapping decapeptides corresponding to the entire sequence of PIA from strain FA19 (8) was synthesized, with adjacent peptides overlapping by five residues. Antibody SM100 reacted strongly with peptide ^{211}AKLTWRNDNS^{220} (optical density [OD], 0.6), weakly with the adjacent peptide ^{221}WRNDNSHNSQT^{230} (OD, 0.08), and with no other peptides (OD, <0.02). A series of hexapeptides were constructed encompassing residues 211 to 221, with adjacent peptides overlapping by five residues. Antibody SM100 reacted strongly with the peptide ^{214}TWRNDN^{219} (OD, 0.7), less strongly with the two adja-

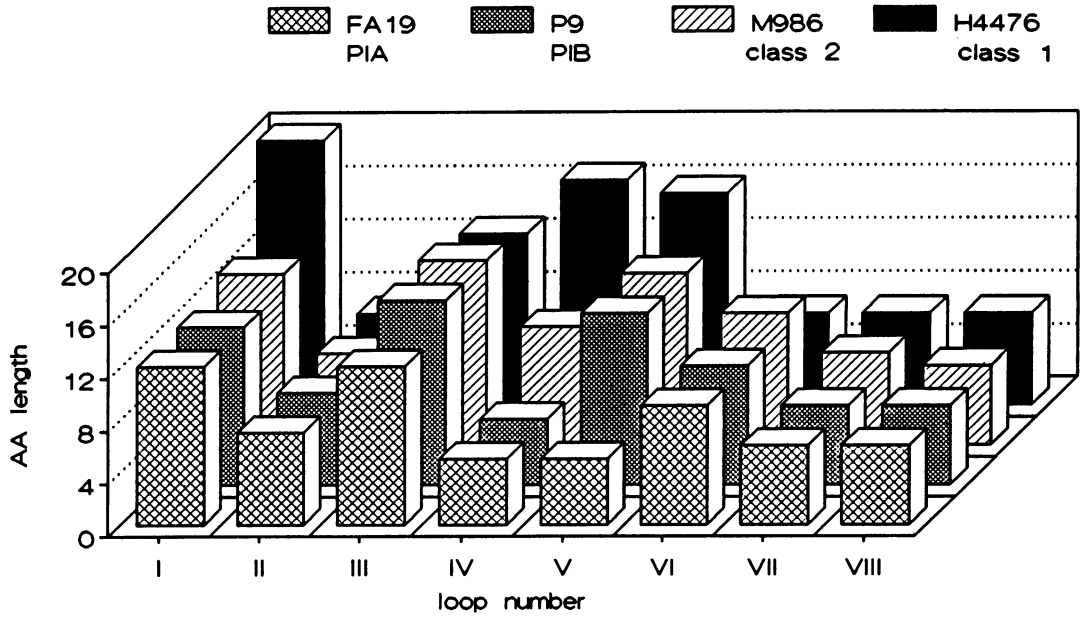

FIG. 4. Comparison of the lengths of the eight surface-exposed loops in class 1, class 2, PIA, and PIB proteins, as predicted by the models shown in Fig. 2. AA, amino acid.

TABLE 1. Antisera against synthetic peptides

Peptide	Loop (protein class-strain)	Sequence[a]	Reactivity in whole-cell ELISA[b]			
			MC50	H44/76	2996	M986
018	1 (1-H44/76)	X G G A Q A A N G G A S G	–	1/300	–	–
032	1 (1-MC50)	X G G N I Q A Q L T E Q P Q V T N G V Q G N	1/2,700	1/300	–	–
030	2 (1-MC50)	X G G V S V A G G G A S Q W G N	–	–	–	
026	3 (1-MC50)	X G G D S N N D V A S Q L G I F K	–	–	–	–
017	4 (1-MC50)	X G G Y Y T K D T N N N L	1/300	1/300	–	–
021	4 (1-2996)	X G G H F V Q Q T P Q S Q P T L	–	–	1/2,700	–
025b	5 (1-MC50)	X A N V G R N A F E L F L I G S A T S D E A K G	1/300	1/900	–	–
024	6 (1-MC50)	X G G L S E N G D K A K T K N S T T E	–	–	–	–
028b	7 (1-MC50)	X G G E R G K K G E N T S Y D Q	–	–	–	–
031	8 (1-MC50)	X K R N T G I G N Y T Q I N A A	–	–	–	–
029	1 (2-M986)	X G G V K D A G T Y K A Q G G K S K T A T Q	1/300	–	1/900	1/300
036	3 (2-M986)	X G G W E S G S N T E D V L G L G	–	–	—[c]	—[c]
037	4 (2-M986)	X G G Y V P R D N A N D V D K Y K H T K S S R E S	–	–	—[c]	—[c]
027	5 (2-M986)	X A D L N T D A E R V A V N T A N A H P V	1/900	–	1/2,700	1/900
038	6 (2-M986)	X G G A A K N N E V G S T K G K K H E Q T	–	–	–	–

[a] X = *N*-(*S*-acetylmercaptoacetyl)-.

[b] A threefold dilution series of each antiserum was prepared, starting with 1/300. Reactivity is expressed as the highest dilution that gave an OD at 450 nm of >0.6. The coat antigen consisted of whole cells of strains MC50 (NT:P1.16), H44/76 (15:P1.7,16), 2996 (2b:P1.2), and M986 (2a:P1.2). With the exception of antiserum against 031, all antisera reacted with the homologous peptide.

[c] —, positive with OMV instead of whole cells.

cent peptides (OD, 0.3 to 0.4), and not at all with the remaining peptides (OD, <0.02), thus defining the optimum epitope recognized as the sequence TWRNDN located at the apex of loop 6.

The epitope for the P1.2-specific bactericidal monoclonal antibody MN4C5E is presumed to lie in loop 4. This is based on the weak reaction (OD, 0.2) of the antibody with the peptide corresponding to this region of strain 2996, which was also used in the immunization experiments for which results are shown in Table 1. In addition, since the original immunoreactive λgt11 clone carried the C-terminal *Eco*RI fragment starting at residue 100 (see Materials and Methods) and since loop 4 contains the only region of major sequence divergence in this part of the protein, it seems very likely that the P1.2 epitope is indeed located there.

DISCUSSION

The model presented in this paper predicts the existence of eight surface-exposed loops in all four classes of neisserial porins studied. By raising antisera against synthetic peptides (Table 1) and mapping epitopes for bactericidal monoclonal antibodies (Table 2), we have confirmed the surface exposure of four loops: loops 1, 4, and 5 for class 1 protein, loops 1 and 5 for class 2 protein, loop 5 for PIB, and loop 6 for PIA. In addition, the epitope for a nonbactericidal monoclonal antibody that binds only weakly to whole cells was mapped to loop 3 of PIB. A comparison of these results with the model suggests that the length of the surface loops is correlated with the ability to generate antibodies against them that also bind to whole cells (Fig. 4). Thus, in class 1 protein the longest loops are 1 and 4, which contain the variable sequences determining subtype specificity. The next longest is loop 5, which is less variable and defined only by antipeptide serum. Since these loops are considerably longer than any other (also compared with the other porin classes), their presence in class 1 protein could explain why antibodies against this protein have the highest bactericidal activity and give the best protection in an animal model system (34, 35). Antibodies against the minor loops 2, 3, 6, and 7 of class 1 protein do not bind to whole cells or OMV. Although this could be due to conformational effects, an alternative explanation is that they are less accessible to antibodies because of shielding effects from other, longer loops or from lipopolysaccharide. With class 2 protein, synthetic peptides corresponding to loops 1 and 5 yielded antisera reactive with whole cells, while those corresponding to loops 3 and 4 bound only to OMV. Possibly, loss of lipopolysaccharide in the sarcosyl extraction step results in increased exposure of these class 2 epitopes, thus causing the observed difference between whole cells and OMV. Loops 1, 3, and 5 are the longest ones in class 2 protein (Fig. 4). In PIB the epitopes for bactericidal monoclonal antibodies are located in loop 5; in PIA, this loop is reduced by 15

TABLE 2. Epitopes for monoclonal antibodies binding to whole cells

Monoclonal antibody(ies)	Protein (specificity)	Loop	Sequence	Reference
MN14C11.6, ADAM1	Class 1 (P1.7)	1	GASGQVKVT	23
MN5C11G, 62-D12	Class 1 (P1.16)	4	TKDTNNNLTL	23
MN3C5C	Class 1 (P1.15)	4	RQNNTDVF	23
MN4C5E	Class 1 (P1.2)	4	HFVQQTPQSQPTL	This study
SM21, SM22, SM203	PIB (type specific)	5	IEYEHQVY	6
SM24	PIB (cross-reacting)	5	SIPS	6
SM198	PIB (cross-reacting)	3	WESGK	6
SM100	PIA (type specific)	6	TWRNDN	This study

residues and the type-specific epitopes map to loop 6. All things considered, it would seem that the longest loops in the model are the most exposed parts of the protein and therefore both the most variable and immunodominant.

Some information on the location of exposed segments in the gonococcal PIA and PIB proteins has been reported previously. The variable nature of the surface-exposed parts of these proteins has been amply demonstrated by the large number of different serovar patterns (21) and also by peptide mapping of surface-iodinated protein (19). Both proteolysis studies of intact gonococci (5, 19) and the analysis of PIA-PIB hybrids constructed by genetic transformation (7) have demonstrated the existence of several distinct, surface-exposed segments. Mostly, these were not located precisely enough to allow a meaningful comparison with the present model, but they do conform to its general outline. The surface-exposed chymotrypsin cleavage site in PIB protein was precisely located by protein sequencing (14). This site is situated within loop 5 of the model.

The structures of the OmpF and PhoE porins from *E. coli* have been studied by a variety of biophysical techniques (18, 32, 42). These studies have shown that they are present in the outer membrane as trimers, with the individual monomers in the form of β barrels. In such a structure, the polypeptide crosses the outer membrane many times, forming a β-sheet structure that folds into a cylinder, with hydrophobic amino acid residues facing outward to the membrane lipids and the hydrophilic ones facing inward into the pore. Extensive genetic studies with mutant and hybrid porins have shown that it is possible to identify exposed and transmembrane segments in the sequence and thus construct topology models (17, 38). Although no biophysical data on the structure of any neisserial outer membrane protein are available, we assumed in the construction of our model that the basic porin structure is the same in both organisms. The following observations support this assumption: (i) some homology between *Neisseria* and *E. coli* outer membrane proteins has been found, i.e., between class 4/PIII and OmpA (20) and to a lesser extent between PIB and the OmpF, OmpC, and PhoE porins (14); (ii) expression of both PII (27) and class 1 (43) protein in *E. coli* resulted in the presence of the protein at the cell surface; and (iii) the general pore characteristics, such as apparent diameter and slight ion selectivity, are very similar for *E. coli* and *Neisseria* porins (39).

The structure of neisserial porins presented in this paper should form a useful working model for further studies of the vaccine potential of these proteins and peptides derived therefrom. In particular, one can now envisage the construction of multivalent proteins by using recombinant DNA techniques to replace minor loops by immunodominant ones from relevant sero(sub)types.

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